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Toxicity of Copper-Spiked Sediments to Freshwater Invertebrates

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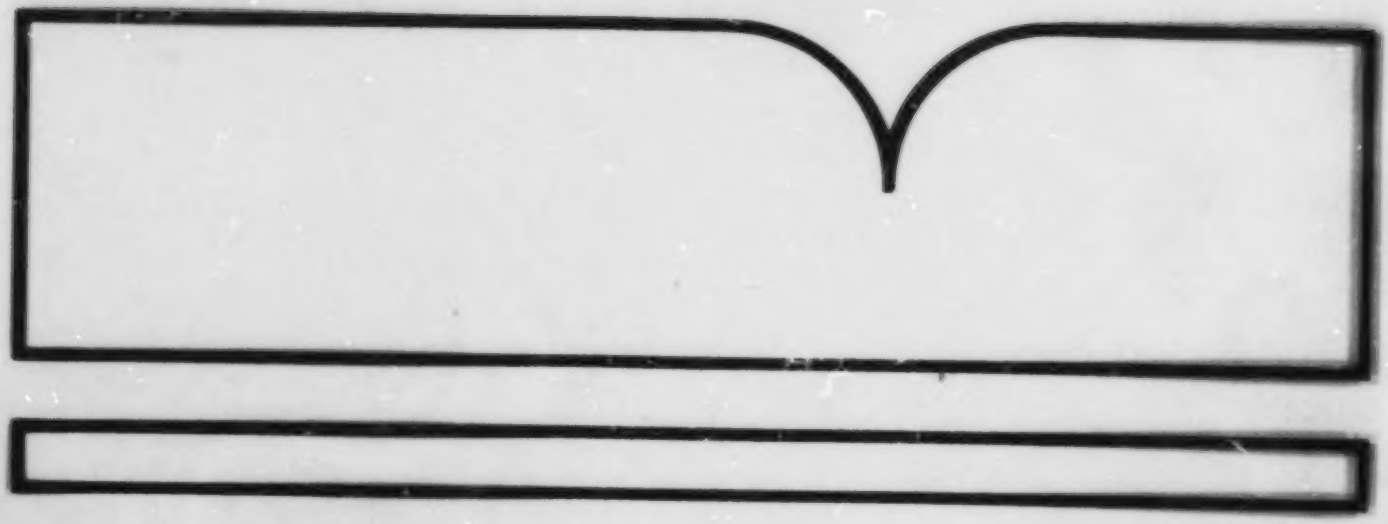

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16. ABSTRACT

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TOXICITY OF COPPER-SPIKED SEDIMENTS TO FRESHWATER INVERTEBRATES

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Abstract — Natural freshwater sediments from two Oregon sites were spiked with copper in the laboratory and two static toxicity tests were conducted with series of copper concentrations ranging from 59 to 10,600 mg/kg of dry sediment. Water (800 ml) was added to 1-liter test beakers over the sediment (200 ml) and repeatedly removed and replaced to lower the aqueous copper concentrations. When copper concentrations stabilized, i.e., attained equilibrium between water and sediment, the midge *Chironomus tentans*, the cladoceran *Daphnia magna* and the amphipods *Hyalella azteca* and *Gammarus lacustris* were introduced as test animals in solid-phase sediment toxicity bioassays. Based on dry-weight sediment copper concentrations, LC_{50} values (48-h for *Daphnia*, 10-d for other organisms) ranged from 681 to 2,296 mg/kg.

Keywords — Sediment Copper Toxicity Invertebrates *Chironomus* *Daphnia* *Hyalella* *Gammarus*

INTRODUCTION

Sediments are known to act as sinks and reservoirs for certain waterborne toxicants in both lotic and lentic systems. These toxic chemicals can affect benthic organisms that ingest particles of contaminated sediment. In addition, toxicants can be released into overlying and interstitial waters by acidification, dredging, oxidation-reduction potential changes, bioturbation, thermal effects, storm events, ship traffic, lake destratification or complexation [1] to affect aquatic organisms.

The environmental effects of toxic materials in sediments are poorly understood. Most pertinent literature addresses effects of dredging [2–4] on the release of toxicants and is usually restricted to marine environments. Biological effects of toxic sediments have been investigated using bulk sediment chemical analyses [5,6], elutriate tests [7] and recirculating bioassay apparatus [8]. Bulk sediment chemical analyses provide inventories of elemental constituents and contaminants but do not consider bioavailability. Elutriate tests assay only the soluble components and fail to address toxicity due to particle ingestion by the benthic community.

The U.S. Environmental Protection Agency (U.S. EPA) has few data on the concentrations of toxicants in sediment which are harmful to benthic animals, although

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the Federal Clean Water Act (P.L. 95-217) contains several sections dealing with sediment quality. Water quality criteria for individual toxicants are based on toxicity and are commonly expressed in terms of concentrations in water. To our knowledge, no study has determined estimates of sediment quality criteria for individual toxicants in freshwater environments.

This article presents results of a study conducted to determine the toxicity of copper-contaminated sediment to several freshwater invertebrates. Sediments obtained from two freshwater sources in Oregon were spiked with different concentrations of copper, repeatedly rinsed to lower the aqueous copper concentrations to equilibrium levels and assayed using the midge *Chironomus tentans*, the cladoceran *Daphnia magna* and the amphipod crustaceans *Hyalella azteca* and *Gammarus lacustris*. Death was used as the acute toxicity endpoint and LC_{50} values were calculated for each sediment from the dose–response relationships.

MATERIALS AND METHODS

Test facilities

Tests were conducted in heavy-duty, washed and acid-rinsed, 1-liter glass beakers, fitted with glass covers. The beakers were contained in stainless steel water baths which controlled the temperature at 19 ± 1°C. The photoperiod was maintained at 16 h light:8 h dark during both tests. Individual glass-tipped Tygon air lines from a polyvinylchloride manifold fed by an oil-free compressor aerated all beakers to maintain dissolved oxygen near saturation levels. Air lines tips were submerged about 2 cm, sufficient to aerate the water but not disturb the sediment.

Sediments

Two sediment types were collected by grab sampling and used for the two tests. Sediment for the first test was from the Tualatin River (TR), located near Durham, 15 km southwest of Portland, OR. It was collected with a Ponar sampler and frozen to kill indigenous animals—primarily midges—prior to testing. TR sediment contains about 1.8% organic carbon and is composed of 40.7% sand, 47.5% silt and 11.8% clay. Sediment from Soap Creek Pond No. 7 (SCP), located on Oregon State University property 12 km north of Corvallis, OR, was collected with an Eckman sampler, frozen and used in the second bioassay. SCP sediment contains 3.0% organic carbon and is composed of 15.2% sand, 28.9% silt and 55.9% clay. These measurements were done by the Oregon State University Soil Science Laboratory using the wet oxidation organic carbon method of Walkley and Black [9] and the hydrometer method of Day [10] for particle size analysis. Wet SCP sediment contained approximately 77% (by weight) moisture while the percent moisture content for TR sediment was not determined.

Dilution water

Tests were conducted at the Western Fish Toxicology Station (WFTS), a facility of the U.S. EPA Corvallis Environmental Research Laboratory. WFTS well water, aerated and temperature-controlled, typically has a median pH of 6.8 (before aeration), mean total hardness of 34 mg/L as $CaCO_3$ and mean total alkalinity of 31 mg/L as $CaCO_3$ [11]. Copper concentrations are at or near detection limits, as are other trace metal concentrations. Water quality characteristics unique to these tests are discussed later in this report. Analytical methods were those recommended in *Standard Methods* [12].

Toxicant

Stock solutions were prepared by dissolving 268 g of reagent-grade cupric chloride ($CuCl_2 \cdot 2\ H_2O$) in 1 liter of reverse-osmosis (RO) treated water, giving a concentration of 0.1 g Cu/ml of stock solution. Samples for aqueous copper analyses were collected in

new 10-ml disposable test tubes and acidified by the addition of 0.1 vol% of concentrated nitric acid. Low-level aqueous copper concentrations (< 50 μg/L) were determined by carbon furnace techniques on a Perkin-Elmer Model 305 B atomic absorption spectrophotometer (AAS) [13]. The detection limit was 5 μg/L. Higher concentrations of aqueous copper (> 50 μg/L) were determined by flame AAS on a Perkin-Elmer Model 403 [13]. The detection limit was 0.1 mg/L. Sediment copper samples were subjected to rigorous acid digestion prior to analysis [14] and were analyzed on the Perkin-Elmer Model 403 AAS [13]. For the total of 79 copper spikes run during this experiment, the average recovery was 99.5 ± 5.9%.

To determine partitioning of copper between liquid and solid phases in the TR test, the percentage of copper associated with each medium was determined. The amount of copper in the water column (total concentration × 0.8 liter of water) was divided by the amount of copper on the sediment (dry weight sediment concentration × weight of sediment) plus that in the water. Because the sediment was not weighed in the SCP test, partitioning could not be determined in that test.

Test organisms

Daphnia used in tests were between 4 and 7 d old, obtained from a WFTS static culture maintained at 19 ± 1°C, 200 mg/L total water hardness and a 16-h light:8-h dark photoperiod. They were fed vitamin-fortified algae (*Selenastrum capricornutum*) and a mixture of Oregon Moist Pellet (OMP) fish food and yeast. Animals were gently pipetted into test beakers without exposure to air. *Chironomus* were late second- or early third-instar larvae, 18 to 20 d old when introduced, obtained from a flow-through WFTS well-water culture and fed *S. capricornutum* and the powdered grass product Cerophyl (Agri-Tech, Kansas City, MO). They were gently transferred to test beakers with a stainless steel hook. *Hyalella* were reared in flow-through aquaria at 10°C and 16-h light:8-h dark photoperiod and were tested when they were larger juveniles and young adults. They were transferred to 13°C water and then to 16°C water over a 2-d period to acclimate them to the test temperature. They were fed soaked maple leaves, wheat, *S. capricornutum*, frozen brine shrimp, Cerophyl and OMP. Animals were transferred to test beakers in a glass pipette, as were daphnids. *Gammarus* were reared at 19°C and a 16-h light:8-h dark photoperiod in flow-through aquaria. Larger juveniles and young adults were used for testing. They were fed the same foods as *Hyalella* and were transferred to test beakers using a small-mesh stainless steel screen formed in the shape of a spoon.

Test procedures and experimental design

Tualatin River. Sediment was collected and frozen for 24 h. Two hundred milliliters of wet sediment was put in each of nine beakers and covered with 800 ml of well water. Three days later, copper was added to eight of the beakers (Table 1) by adding copper stock solution to the water at levels of from 50 to 6,400 mg Cu per beaker. No copper was added to the control beaker, which contained a background level of 59 mg Cu/kg of dry sediment. Contents of all beakers were mixed for 5 min with a stir bar, facilitating the sorption of copper by the sediments. Beginning 3 d after copper introduction and continuing for 42 d, all sediments were "washed" with well water approximately every 5 d. Aqueous total copper was measured before each wash. Washing involved draining most of the overlying water, refilling and stirring. All beakers were regularly topped off with RO water to compensate for evaporative losses.

One week after the last wash, aqueous total copper concentration was again determined, to assure that copper equilibrium between water and sediment had been reached

Table 1. Copper concentrations in sediment and water, and survival of test organisms for Tualatin River test

Beaker no.	Dry weight of sediment (g)	Copper added (mg)	Total copper removed[a] (mg)	Copper remaining[b] (mg)	Copper removed (%)	Copper in sediment[c]	Mean total aqueous copper[d] (mg/L)	pH	Number alive/total[e]	
									Chironomus	*Daphnia*
1	156.78	0	0.7	9	7	59	0.006	7.6	10/10	9/10
2	113.26	50	0.5	45	1	400	0.022	7.6	10/10	9/10
3	117.07	100	0.8	126	1	1,080	0.103	7.4	9/10	5/10
4	136.24	200	3	245	1	1,800	0.160	7.4	4/10	0/10
5	102.41	400	30	404	7	3,950	0.200	7.3	4/10	0/10
6	162.32	800	108	722	13	4,450	0.445	7.0	3/10	0/10
7	156.06	1,600	564	1,194	32	7,650	1.60	6.6	1/10	0/10
8	158.47	3,200	1,931	1,406	58	8,870	4.35	6.1	0/10	0/10
9	108.67	6,400	5,336	1,152	82	10,600	9.20	5.7	0/10	0/10

[a]Each amount represents a total of eight washings.
[b]Derived by multiplying mg Cu/kg sediment by weight (kg) of sediment present.
[c]mg Cu/kg of dry sediment.
[d]Mean of two measurements during exposure, one on day 1 and one on day 10.
[e]*Chironomus* exposed for 10 d and *Daphnia* exposed for 48 h.

in each beaker, and 10 *Chironomus* per beaker were introduced for a 10-d exposure. The midges were fed a total of six drops of a 1 g Cerophyl/10 ml water slurry during the test. On the eighth day of the midge exposures, 10 *Daphnia* per beaker were introduced for a 2-d exposure. At termination on day 10, total aqueous copper was again analyzed, sediment samples were collected for dry weight copper determinations and the oven-dried weight of the sediment in each beaker was measured.

Soap Creek Pond. Two hundred milliliters of wet sediment, frozen for 24 h, was put into each of 18 beakers (six treatment levels with three beakers per treatment level) and covered with 800 ml of well water. Each beaker was spiked with an appropriate quantity of copper stock solution and manually stirred with a glass rod for 1 min. Amounts ranging from 37.5 to 600 mg Cu per beaker were added to 15 beakers; three control beakers received no additional copper but contained mean background levels of 210 mg Cu/kg of dry sediment (Table 2). A 200-ml sample of wet sediment was oven-dried for 3 d at 54°C to determine percent moisture content. One, 3 and 7 d later, all sediments were washed and stirred and aqueous copper was measured to determine concentration equilibria.

After the last washing, total aqueous copper was again measured to assure stabilization, and 10 *Gammarus*, 15 *Hyalella* and 15 *Chironomus*, respectively, were introduced into each of the three separate beakers at each treatment level. All animals were fed a small amount of newly hatched brine shrimp during the test. On the third day of exposure, total and soluble aqueous copper were measured. Soluble copper was measured after centrifuging at 10,000 rpm for 10 min and then acidifying the supernatant. On the eighth day of exposure, dissolved oxygen was measured in all beakers and 20 *Daphnia* were introduced into each of the beakers containing midges. On the ninth day of exposure, total hardness was measured in all beakers. On the tenth day, all surviving animals were counted and total and soluble aqueous copper were determined, as were turbidity and pH. Sediment was then collected for dry-weight copper analyses.

A preliminary range-finding test was conducted with SCP sediment to determine copper levels lethal to *Chironomus* and *Daphnia*. The lowest concentration (3,570 mg/kg) killed all the test organisms. Dry sediment in each beaker was weighed in this preliminary test. Weights ($n = 11$) ranged from 49.28 to 57.80 g and averaged 54.35 ± 2.34 g. This average weight was used in calculating the approximate weight of copper remaining per beaker in the definitive SCP test (Table 2).

Our experimental design does not meet commonly recommended replication requirements for acute toxicity tests without sediments [15] but it is adequate for establishing trends and understanding mechanisms of toxicity in sediment tests.

Statistics

LC_{50} values (48-h for *Daphnia*, 10-d for other animals) and their 95% confidence intervals were determined by regressing dry-weight copper concentrations in the sediment, total aqueous copper concentrations and soluble copper concentrations versus mortality for each test species, using the Trimmed Spearman-Karber method [16].

To demonstrate that aqueous copper concentrations remained relatively constant during the tests, measured concentrations in each beaker were compared at the beginning and end of each bioassay. These comparisons enabled us to determine if equilibria had indeed been achieved before the exposures began.

Table 2. Copper in sediment and water, and survival of test organisms for Soap Creek Pond test

Beaker no.	Copper added (mg)	Total copper removed[a] (mg)	Approximate copper remaining[b] (mg)	Copper removed (%)	Copper in sediment[c]	Mean total aqueous copper[d] (mg/L)	Mean soluble aqueous copper[e] (mg/L)	pH	Turbidity (NTU)	Number alive/total[f]			
										Gammarus	*Hyalella*	*Chironomus*[g]	*Daphnia*[g]
1	0	0.3	12	2	213	0.082	0.014	7.4	1,740	9/10			
2	0	0.1	12	1	217	0.024	0.008	7.8	290		15/15		
3	0	0.1	11	1	201	0.019	0.005	7.9	200			11/15	15/20
4	37.5	0.4	26	1	488	0.860	0.038	7.2	2,820	10/10			
5	37.5	0.4	34	1	618	0.460	0.024	7.6	830		13/15		
6	37.5	0.3	30	1	562	0.220	0.025	7.7	330			13/15	14/20
7	75	0.7	51	1	936	1.26	0.066	7.1	1,390	6/10			
8	75	0.4	48	1	875	0.69	0.029	7.0	850		13/15		
9	75	0.6	45	1	823	0.43	0.037	7.3	500			8/15	7/20
10	150	3.3	88	4	1,620	1.19	0.074	7.1	440	0/10			
11	150	3.7	100	4	1,850	1.35	0.073	6.5	390		0/15		
12	150	2.9	84	3	1,550	0.88	0.069	6.4	315			1/15	0/20
13	300	19.6	164	11	3,010	1.42	0.16	6.9	118	0/10			
14	300	20.0	139	12	2,560	1.12	0.45	6.0	64		0/15		
15	300	24.2	159	13	2,920	1.63	0.38	6.3	145			0/15	0/20
16	600	119	228	34	4,190	3.27	2.70	5.9	7	0/10			
17	600	120	221	35	4,070	3.70	3.60	4.9	2		0/15		
18	600	113	235	32	4,320	3.90	3.70	5.2	1			0/15	0/20

[a]Each amount represents a total of three washings.
[b]Derived by multiplying mg Cu/kg dry sediment by 0.05435 kg of sediment (approximate weight of sediment per beaker).
[c]mg Cu/kg of dry sediment.
[d]Mean of three measurements during exposure, one on day 1, one on day 4 and one on day 10.
[e]Mean of two measurements during exposure, one on day 4 and one on day 10.
[f]*Daphnia* exposed for 48 h, other animals exposed for 10 d.
[g]*Chironomus* and *Daphnia* in same beakers.

RESULTS

Tualatin River

The TR test showed a graded dose–response relationship for survival of both midges and cladocerans (Table1). The 10-d LC_{50} (95% confidence limits) for *Chironomus* was 2,296 (1,690–3,119) mg Cu/kg dry sediment. The 48-h LC_{50} (95% confidence limits) for *Daphnia* was 937 (679–1,291) mg Cu/kg dry sediment. Copper concentrations and pH were inversely proportional (pH attaining a low of 5.7), although neutral to slightly alkaline pH was associated with mortality-effect concentrations, e.g., beakers 3 to 6.

Total aqueous copper concentrations, measured on days 1 and 10, increased an average of 6% during the bioassay. Excluding the control beaker, the concentrations increased an average of only 4% during the test, indicating that copper was indeed near equilibrium between the sediment-bound and aqueous phases. LC_{50} values based on soluble copper concentrations were not calculated because only total aqueous copper concentrations were measured.

Soap Creek Pond

A graded dose–response relationship was exhibited in the SCP test as well (Table 2). LC_{50} values (95% confidence intervals), in mg/kg based on dry-weight sediment copper concentrations, were 964 (777–1,196) for *Gammarus*, 1,078 (922–1,259) for *Hyalella*, 857 (685–1,073) for *Chironomus* and 681 (478–969) for *Daphnia*. Based on soluble copper concentrations, LC_{50} values (95% confidence limits) in μg/L were 61 (54–70) for *Gammarus*, 39 (34–46) for *Hyalella*, 38 (31–48) for *Chironomus* and 30 (18–49) for *Daphnia*.

Dissolved oxygen averaged 9.1 ± 0.1 mg/L and total hardness averaged 57 ± 18 mg/L as $CaCO_3$ during this test. Neither parameter was correlated with the species of organism present or copper concentration. Turbidity was influenced both by the species present and the copper concentration (Table 2). Turbidity averaged 1,086, 404 and 248 nephelometric turbidity units (NTU) in beakers containing *Gammarus*, *Hyalella* and *Chironomus*/*Daphnia*, respectively. Turbidity averaged 743, 1,327, 913, 382, 109 and 3 NTU, going from low to high concentration (and from slightly alkaline to acidic pH). Copper concentrations and pH were inversely proportional (pH attaining a low of 4.9), although effect-concentrations were associated with neutral to slightly alkaline pH, e.g., beakers 4 to 9.

Total aqueous copper concentrations, measured on days 1 and 10, increased an average of 28% during the bioassay. Excluding the control beakers, the total concentrations decreased an average of 5% during the test. Soluble copper concentrations, measured on days 3 and 10, increased an average of only 11% during this period, and copper concentrations were indeed near equilibrium between solid and liquid phases.

Table 3 summarizes the differences between test sediments used in the research as well as calculated LC_{50} values based on dry-weight sediment copper concentrations, total aqueous copper concentrations and soluble copper concentrations.

DISCUSSION

All the observed toxicity in the SCP copper-spiking test can be accounted for by soluble copper, because the LC_{50} values are very similar to those obtained in copper (in water only) bioassays. Little, if any, of the toxicity can be attributed to sediment-bound copper. Static copper bioassays (water only) demonstrated that 10-d LC_{50} values for *Hyalella* and *Gammarus* were 59 and 31 μg/L, respectively (A.V. Nebeker et al., unpublished data). In the SCP sediment test, beakers 7 to 9, an average of 48 mg of copper was present in each 1-liter test container of sediment and water (Table 2). However, of the 48 mg Cu, only approximately 35 μg was in a dissolved form (44

Table 3. Summary of sediment types and LC_{50} (2-d for *Daphnia*, 10-d for other organisms) calculations from copper-spiking sediment toxicity tests

Sediment	% organic carbon	% sand	% silt	% clay	Test organism	LC_{50} (95% confidence intervals) based on: Dry weight of sediment Cu (mg/kg)		Total aqueous Cu (μg/L)		Soluble Cu (μg/L)	
Tualatin River	1.8	40.7	47.5	11.8	*Chironomus tentans*	2,296	(1,690 – 3,119)	249	(158 – 394)	—[a]	
					Daphnia magna	937	(679 – 1,291)	77	(49 – 119)	—[a]	
Soap Creek Pond	3.0	15.2	28.9	55.9	*C. tentans*	857	(685 – 1,073)	428	(312 – 587)	38	(31 – 48)
					D. magna	681	(478 – 969)	299	(141 – 633)	30	(18 – 49)
					Gammarus lacustris	964	(777 – 1,196)	1,152	(1,081 – 1,228)	61	(54 – 70)
					Hyalella azteca	1,078	(922 – 1,259)	719	(527 – 980)	39	(34 – 46)

[a]Soluble copper was not measured.

μg/L), and only partial mortality of all test species occurred. LC_{50} values calculated for dissolved copper (in the SCP test) for the four test species ranged from 30 to 61 μg/L, which agrees well with LC_{50} concentrations determined in water without sediment. Toxic effects apparently occur when copper is mobilized from the solid (sediment) phase to the liquid (dissolved) phase. The relative distribution of copper, or any other contaminant, between the two phases is controlled by equilibrium kinetics [1]. Therefore, higher sediment contaminant concentrations result in higher dissolved contaminant concentrations, which in turn lead to greater biological impacts. The solid-phase LC_{50} values for copper, therefore, are simply the sediment copper concentrations that correspond to LC_{50} values for dissolved copper (liquid phase) at equilibrium for a given sediment type. This distinction may be academic, except for the issue of whether or not water quality standards are adequate to deal with problems of sediment contamination. If we know that toxic effects of sediment contaminants are only exerted after the contaminants are mobilized from the solid to liquid phase, then perhaps water quality standards applied in the right location, i.e., near the sediment water interface or in interstitial waters, may still adequately protect aquatic life.

The LC_{50} values for copper in the TR sediment (solid phase) were 1.4 and 2.7 times greater for *Daphnia* and *Chironomus*, respectively, than for the SCP sediment. Such differences are to be expected and probably reflect the composition of the two sediments and the ability of the sediment constituents to bind and hold copper. If one were interested in determining LC_{50} values for a sediment contaminant at a particular site, sediments having characteristics similar (i.e., organic carbon, sand, silt and clay content) to those at the site should be used to minimize the possibility of markedly different responses.

The LC_{50} values for copper in sediment that we have reported correspond reasonably well to copper concentrations that have been shown to adversely affect natural benthic communities. Kraft and Sypniewski [17] found significant effects of copper on benthic macroinvertebrate density and number of taxa present in Keweenaw Waterway, MI, which is polluted by copper tailings, when sediment copper concentrations ranged from 39 to 1,229 mg/kg (mean 589 mg/kg). *Hexagania* sp. were very sensitive to copper, as were mollusks, amphipod crustaceans (including *Gammarus* sp.), caddisflies, oligochaetes and several chironomid midges. However, two chironomids were very tolerant of sediment copper. Sediment copper concentrations that killed 50% of the animals in our study (681–2,296 mg/kg) are only slightly higher than those measured by Kraft and Sypniewski [17]. Longer exposure times and acclimation to toxicants under field conditions, differences in sediment composition and exposure of more sensitive life stages could easily explain the differences between laboratory and field data.

Species-specific sensitivity to soluble copper (determined in the SCP test), in order of decreasing sensitivity, was *Daphnia, Chironomus, Hyalella* and *Gammarus*. Control mortality varied from 0 to 27% in these sediment tests. High control mortality may have been due to predation of weakened *Daphnia* by *Chironomus* or to difficulty in finding organisms at test termination because of turbidity and midge burrowing.

In choosing an ideal test organism, the availability and rearing requirements as well as species- and toxicant-specific differences in toxicity must be considered. The amphipods, although apparently less sensitive in this copper-spiking study, may be more sensitive to other toxicants. Also, the degree of intimate association with the sediment must be taken into account. Daphnids are zooplankton, which are predominantly associated with the water column. They seem to

be more sensitive to copper than *Chironomus* or *Hyalella*, which burrow into the sediment. We recommend using an array of test organisms that represent several niches.

Turbidity in the SCP test was highest in beakers containing the most active animals, e.g., *Gammarus*, because they stirred the sediment into the water column. Turbidity was lowest in beakers containing chironomids and daphnids, and those showing acidic pH and higher levels of copper. The sediment in these beakers contained high levels of iron (qualitatively determined in precipitates) and the beakers were under continuous aeration during the study. Under these conditions, the formation of iron oxide and hydroxide precipitates would occur at higher pH levels. Conversely, the hydrolysis of the higher amounts of added cupric chloride would lower pH values and inhibit formation of these iron precipitates. However, animal mortality was not directly influenced by pH effects because beakers containing just enough copper to kill all test organisms had neutral pH.

Greater than 99% of the copper in TR was partitioned into the solid phase, either chemically or physically bound to particles. However, copper in the interstitial water, which was not monitored in this study, contributed a small amount to the sediment-sorbed copper because only overlying water was removed prior to analyzing the sediments. Due to the lower clay and organic carbon content (and therefore the complexing capacity) of the TR sediment, one would expect the SCP sediment to sorb more copper.

In the SCP test, between 4 and 97% of the water column copper was in a soluble form, depending on copper concentration and pH. Higher percentages were associated with the higher total copper concentrations. At the LC_{50} concentrations, only about 5 to 10% was soluble copper. Furthermore, some soluble copper exists in forms other than ionic copper. For instance, humic substances can be major copper complexing agents. Levels of copper remaining in solution are directly dependent on water chemistry. Ionic copper is more soluble in low pH waters. Various copper complexes and precipitates are generally not toxic and tend to mask and remove the toxicity ascribed to copper [18]. Therefore, it may be more appropriate to consider soluble ionic copper concentration rather than total recoverable copper.

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